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**Age-dependent elastin degradation is enhanced in chronic obstructive pulmonary disease**

Jeffrey T.J. Huang<sup>1</sup>, Charlotte E. Bolton<sup>2</sup>, Bruce E. Miller<sup>3</sup>, Ruth Tal-Singer<sup>3</sup>, Roberto A. Rabinovich<sup>4</sup>, Colin Palmer<sup>1</sup>, Neil C.Thomson<sup>5</sup> and William MacNee<sup>4</sup>; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) Investigators.

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**Abbreviation:** COPD: Chronic obstructive pulmonary disease, DES/IDES: desmosine and isodesmosine

**Abstract**

Elastin turnover increases with chronological age and COPD accelerates this process beyond normal ageing.

**To the Editor,**

Chronic obstructive pulmonary disease (COPD) is primarily a lung condition characterized by the presence of persistent airflow limitation resulting from inflammation, remodeling of small airways, and emphysema. It is well-recognized that the impacts of COPD extend beyond the lung with many patients suffering systemic manifestations such as cardiovascular diseases that affect morbidity and mortality[1]. “Accelerated ageing” has been proposed as a mechanism that underlies many of the pulmonary and extra-pulmonary consequences of COPD[2, 3]. It is thought that a decline in organ function is a feature of ageing in response to the accumulation of cell and molecular damage, and in the case of COPD, noxious inhalants such as tobacco smoke increase this damage, thus accelerating the ageing process, leading to the development of COPD. With the exception of lung function decline, however, evidence indicating that tobacco smoking or COPD accelerates age-associated deterioration remain scarce.

The degradation of elastin, a key protein component of connective tissues that critically provides the characteristics of elasticity, resilience, and deformability, is an important feature in normal ageing and in COPD. Elastin has a long half-life (~74 years[4]) in contrast to minutes to days for most intracellular proteins[5]. This longevity increases its susceptibility to oxidative and chemical damage, which are believed to drive age-related elastic fiber turnover associated with low-grade chronic inflammation. This turnover can be measured by the levels of circulating desmosine and isodesmosine (DES/IDES), two crosslinking moieties that specifically exist in mature elastin[6]. We and others have shown increased circulating DES/IDES levels in COPD patients in comparison to healthy smokers and never-smokers[7-10]. Recently, we further demonstrated that this increase was associated with higher mortality and cardiovascular morbidity in a large cohort study[10]. Interestingly,

among all of the demographic variables analyzed, circulating DES/IDES levels display the strongest and consistent correlation with chronological age in three independent cohorts of COPD patients[10, 11]. This marker also showed a stronger association with age than inflammatory markers (e.g. fibrinogen, Clara cell secretory protein 18, and high sensitivity C reactive peptide)<sup>10</sup>. These observations raise the possibility that systemic elastin turnover is an age-dependent process, and that COPD and smoking can alter the rate of this process.

To test the hypothesis, we analyzed data collected from three study cohorts previously described<sup>10,11</sup> comprising 261 never-smoker controls, 380 smoker controls, and 1,332 COPD patients. The relationship between circulating DES/IDES levels and age was analyzed separately in each group. Consistent with the observations from previous studies[10-12], we confirmed that circulating DES/IDES levels were significantly correlated with age in all groups ( $p<0.0001$ , **Figure 1A**). The correlation was weaker in the smoker control group compared to the never-smoker control or COPD group ( $r=0.41$ ,  $0.28$ , and  $0.41$ , for never-smoker control, smoker control and COPD groups, respectively,  $p<0.0001$  for all groups). The positive associations remain highly significant taking into account gender, FEV<sub>1</sub> or pack-years smoking history ( $p<0.0001$ ).

To investigate whether the COPD or smoker control group has a higher age-associated increase in elastin turnover, we compared the slopes of the regression lines between the three groups using a linear mixed effect model incorporating subject-specific random effect, and taking into account of the effect of two visits from some individuals. We found that the slope of elastin turnover to age regression was greater in COPD patients (6.6ng/L per year) compared to never-smokers (2.9 ng/L per year,  $p<0.0001$ ) or smokers (3.1 ng/L per year,  $p<0.0001$ , **Figure 1B**). Further subgroup analysis indicates that smoking status, or its

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51 It is worth noting that circulating DES/IDES and other biomarkers of ageing such as telomere  
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53 length are different in nature, although they are all correlated with chronological age.  
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other typical biomarkers of ageing such as telomere length may be more representative of the cumulative damage over time (i.e. biological age). It is possible that a biologically older individual can have a low ageing activity or vice versa. Thus the potential utility of these biomarker may be different. From an intervention point of view, for example, it may be more logical to target those who are biologically young, but have higher ageing activity, than those who are biologically old but with low ageing activity. Conversely, it may be more sensible to measure biological age to determine long-term progression in response to an intervention.

Quantifying circulating DES/IDES may therefore represent a potential tool for monitoring normal ageing and accelerated ageing, especially in a setting of therapeutic or life style interventions. One of example comes a study of tiotropium bromide which was shown to reduce plasma DES/IDES levels in a small cohort of COPD patients[13]. This drug is also known to be associated with a lower probability of major and fatal cardiovascular events in a COPD population compared to the placebo group[14].

We acknowledge several limitations in the current study. First, we assume the circulating DES/IDES levels represent the rate of proteolytic enzyme activity towards mature elastin. The influence of renal excretion is not taken into account, although our previous analysis did not show an association between renal function and circulation DES/IDES levels[10]. Second, the control subjects were not followed up long enough to observe any meaningful clinical outcomes. Third, while we did not observe an age-dependent acceleration of elastin turnover in the smoker control group, we cannot exclude a possibility of a selection bias that these smokers are more resistant to the development COPD and hence enhanced elastin degradation with age, due to genetic or environmental factors.

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3 In summary, we demonstrated that elastin turnover increases with age in never-smoker  
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5 controls, and COPD patients. The age-related amplification is enhanced in COPD but not in  
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7 smokers without COPD. These results suggest that early intervention in an at-risk population  
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9 should be a priority in reducing ageing-associated morbidity and mortality. Circulating  
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FIGURE LEGENDS

**Figure 1. Modeling of elastin turnover as a function to age in COPD patients, smoker and never-smoker controls.**

- A. Scatter plots of circulating DES/IDES levels and age in never-smoker controls, smoker controls and patients with COPD from three cohort studies.
- B. A predicted linear mixed model of circulating DES/IDES levels as a function of age in never-smoker controls (in blue), smoker controls (in green) and patients with COPD (in red). The equation of the regression lines are shown in the graph with dash line showing 95% confidence. The correlation coefficients rho are 0.41, 0.28 and 0.41, for smoker, never-smoker control and COPD groups, respectively ( $p<0.0001$ ).

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### Abstract

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To test the hypothesis, we analyzed data collected from three study cohorts previously described<sup>10,11</sup> comprising 261 never-smoker controls, 380 smoker controls, and 1,332 COPD patients. The relationship between circulating DES/IDES levels and age was analyzed separately in each group. Consistent with the observations from previous studies[10-12], we confirmed that circulating DES/IDES levels were significantly correlated with age in all groups ( $p < 0.0001$ , **Figure 1A**). The correlation was weaker in the smoker control group compared to the never-smoker control or COPD group ( $r = 0.41, 0.28$ , and  $0.41$ , for never-smoker control, smoker control and COPD groups, respectively,  $p < 0.0001$  for all groups). The positive associations remain highly significant taking into account gender, FEV<sub>1</sub> or pack-years smoking history ( $p < 0.0001$ ).

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These results strongly support the hypothesis that elastin turnover increases with age in general, and that COPD accelerates the age-dependent increase on elastin turnover. However, it does not suggest a greater age-related increase in smoker controls, compared with never-smokers. Overall, these results are in line with the general concept of “accelerated ageing” in COPD.

While circulating DES/IDES can come from any elastin expressing tissue, we speculate that vascular system is the most predominant source due to its size and proximity to blood. The consequence of such elevation in elastin turnover is likely to affect the vascular physiology and cardiovascular outcomes. Indeed, we have shown that the increased circulating DES/IDES levels are associated with higher mortality and cardiovascular comorbidity in COPD[10]. The circulating DES/IDES levels did not, however, relate to emphysema progression or  $\text{FEV}_1$  decline in COPD[10], potentially due to the reasons mentioned previously.

It is worth noting that circulating DES/IDES and other biomarkers of ageing such as telomere length are different in nature, although they are all correlated with chronological age. Because circulating DES/IDES levels estimate the activity of proteolytic enzymes towards mature elastin, it may be more relevant to ageing activity at the sampling time, whereas

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In summary, we demonstrated that elastin turnover increases with age in never-smoker controls, and COPD patients. The age-related amplification is enhanced in COPD but not in smokers without COPD. These results suggest that early intervention in an at-risk population should be a priority in reducing ageing-associated morbidity and mortality. Circulating DES/IDES may represent a tool to monitor the rate of ageing, particularly in identifying high-risk smokers and COPD patients.

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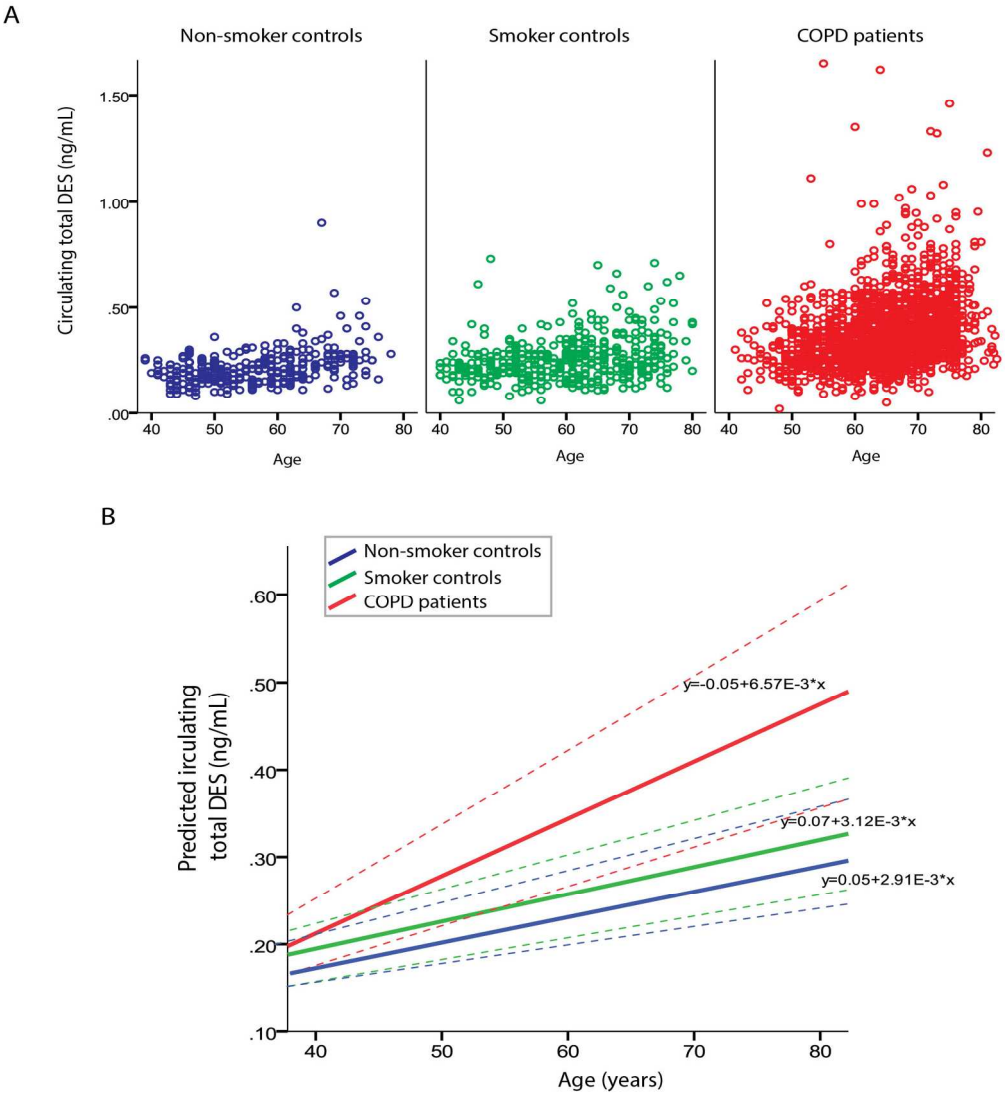
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**FIGURE LEGENDS**

**Figure 1. Modeling of elastin turnover as a function to age in COPD patients, smoker and never-smoker controls.**

- A. Scatter plots of circulating DES/IDES levels and age in never-smoker controls, smoker controls and patients with COPD from three cohort studies.
- B. A predicted linear mixed model of circulating DES/IDES levels as a function of age in never-smoker controls (in blue), smoker controls (in green) and patients with COPD (in red). The equation of the regression lines are shown in the graph with dash line showing 95% confidence. The correlation coefficients rho are 0.41, 0.28 and 0.41, for smoker, never-smoker control and COPD groups, respectively ( $p < 0.0001$ ).



168x183mm (300 x 300 DPI)